Technische Universiteit
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iGEM TU/e 2016
Biomedical Engineering

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PCR Amplification

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1 PCR Amplification

Estimated bench time: 30 minutes
Estimated total time: 2 hours
Purpose: Amplification of DNA with the possibility of expanding the DNA sequence at the beginning and/or end with the primers.
It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.1 Materials

- Autoclaved H_2O
- Autoclaved PCR tubes
- Bucket with ice
- DNA to be amplified
- Forward primer
- Pipettes and tips
- Reverse primer
- 5x phusion buffer
- Phusion DNA polymerase
- Thermal cycler

1.2 Setup & Protocol

- Construct 3 PCR mixtures in the following way:

Component	Quantity/mass/final concentration	Volume (µL)
DNA	30 ng (stock n ng/uL)	30/n
Primer FW	0.5 uM (10 uM stock)	2.5
Primer RW	0.5 uM (10 uM stock)	2.5
5x Phusion buffer	1x	10
dNTPs	200 uM (10 mM stock)	2.5
Phusion DNA polymerase	0.02 U/uL (2 U/uL stock)	0.5
H2O		30-30/n
Total		50

- Use PCR tubes
- Add all components, end with polymerase (transport in cold block)
- Mix well by pipetting up and down.
- Run the following PCR program:

Step	**Temperature (°C)**	**Time (sec)**	**Cycles**
Denaturation	98	30	1
Denaturation Annealing* **Extension***	98 … 72	10 15 .. (20 sec/kb)	30
Final extension	72	600	1
Cooling	4	hold	1

*differs for each PCR dependent on Tm primers

The annealing temperature can be calculated for the set of primers using New Englan Biolabs Tm calculator. An annealing temperature of 3°C lower than the lowest melting temperature was used to increase yields.